In vitro selection of $NaHCO_3$ tolerant cultivars of *Morus alba* (Local and Sujanpuri) in response to morphological and biochemical parameters

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ABSTRACT: *In vitro* experiments were conducted to study the effect of $NaHCO_3$ (alkalinity) stress on saplings of *Morus alba* (cv. Local and Sujanpuri) cultured from nodal explants. For shoot multiplication 2.5 mg/l of 6-benzylaminopurine (BAP) with 0.3 mg/l gibberellic acid (GA_3) were used and root formation was induced with 1.0 mg/l of indolebutyric acid (IBA). $NaHCO_3$ salt was added to the culture medium in three concentrations, i.e. 3.57, 20.0 and 59.0mM that increased pH to 6.2, 7.2 and 8.2, respectively. The increased salt concentration affected survival and growth parameters, subsequent cultures promoted them. The cultured biomass was analyzed for proline, protein, sugars and chlorophyll content. The results indicate an increase of proline, protein and sugars; however, they declined at higher concentrations of $NaHCO_3$. A decrease of chlorophyll was observed at all stress regimes.

Keywords: $NaHCO_3$; biochemical parameters; *in vitro* selection; *Morus alba*

Plants are subjected to a variety of stresses and show a rapid molecular response to changing environmental conditions such as extreme temperature, UV-B radiation, drought, salinity, alkalinity stress etc. (Vashisht, Tuteja 2006). Globally 20% of irrigated land and 2.1% of dry land agriculture suffers from the salt problem (FAO 2000). High concentrations of salts account for a large yield decrease of a wide range of crops all over the world (Yildirim et al. 2006). Therefore it is essential to develop the stress-tolerant cultivars.

Salt tolerance in plants is a complex phenomenon that involves morphological and developmental changes as well as physiological and biochemical processes. Two components have been identified as the probable cause of salt toxicity, namely osmotic stress and ion toxicity. The osmotic stress is associated with a lack of cell-wall extension and cell expansion, leading to cessation of the growth. The ionic effect includes interference with nutrient imbalance, nitrogen uptake, interference with transport of essential ions within the plant, and a lowering of net photosynthetic rates in the affected plants (Greenway, Munns 1980).

One of the most common stress responses in plants is an overproduction of different types of compatible organic solutes (Serraj, Sinclair 2002). Compatible solutes are of low molecular weight, highly soluble compounds that are usually nontoxic at high cellular concentrations. Generally, they protect plants from stress through different courses, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins. Furthermore, because some of these solutes also protect cellular components from dehydration injury, they are commonly referred to as osmoprotectants. These solutes include proline, sucrose, polyols, trehalose and quaternary ammonium compounds (QACs) such as glycine betaine, alaninebetaine, prolinebetaine, choline *O*-sulfate, hydroxyprolinebetaine, and pipecolatebetaine (Rhodes, Hanson 1993). Although much effort has been devoted to genetically engineer plants for overproduction of various osmoprotectants, there has been little success in achieving desired protective levels of these osmolytes in plants (Ashraf, Foolad 2007). Exogenous application of various organic solutes increased the resistance to abiotic stresses. This approach significantly contributes to an increase of crop production in stress environments.

Genetic variability within a species is a valuable tool in screening for higher salt tolerance. *Morus alba* belongs to the family *Moraceae*; it is a multipurpose woody perennial, deciduous and dioecious tree and is purposely cultivated for sericulture. It requires slightly acidic soil (pH 6.2–6.8) and is not easily cultivated on saline/alkaline soils. Due to the heterozygous nature of mulberry plants it is difficult to bring variability through plant breeding and other methods; hence, to create such variability tissue culture might be used.

MATERIALS AND METHODS

Explant collection and sterilization

The healthy nodal segments (1.5 to 2.0 cm) with axillary buds of *Morus alba* (cv. Local and Sujanpuri) collected from 3-year old mulberry plants growing on normal soil (pH = 7.5, EC_e = 0.110 mmhos/cm) at Micromodel, IIT, Delhi, were washed and sterilized with 0.7% (w/v) bleach solution (sodium hypochlorite, $NaOCl_2$) for 10 min and in 0.1% (w/v) aqueous $HgCl_2$ (mercuric chloride) solution for 10 min. Then they were rinsed 5–6 times with sterilized distilled water.

Shoot multiplication

The Murashige and Skoog's (1962) medium containing 3% (w/v) sucrose, 2.5 mg/l of 6 benzylaminopurine and 0.3 mg/l GA_3 was used with freshly cut explants for shoot multiplication. To induce alkaline stress, the medium was supplemented with 3.57, 20.0 and 59.0mM of $NaHCO_3$ increasing the pH to 6.2, 7.2 and 8.2, respectively, before gelling it with 0.8% (w/v) agar. 20 ml of the molten media per 50 ml test tube was autoclaved at 15 psi for 15 min and sterilized explants were inoculated vertically onto the culture medium.

For each treatment twenty explants, one explant per test tube, were maintained and each subculturing was done after 25 days. The first culturing was done on MS + BAP + GA_3 + $NaHCO_3$, followed by subculturing on (*i*) MS + BAP + GA_3, (*ii*) MS + BAP + GA_3 + $NaHCO_3$ (for shoot development) and (*iii*) MS + IBA (for root development). All cultures were maintained in 25 ± 1°C under 16 h photoperiod. For all cultures data regarding survival rate and length of shoots per explant, fresh and dry mass were recorded.

Root induction

Well developed shoots (5.0 cm long) with 3 to 4 leaves were transferred on MS medium supplemented with 1.0 mg/l of indolebutyric acid (IBA) and data on root formation, number and length of roots per plantlet were collected.

Chlorophyll estimation

Chlorophyll content was determined with Hiscox and Israelstam (1979) method using DMSO as a blank.

Estimation of proline

Proline concentration was determined using the method of Bates et al. (1973). Fresh leaves (0.5 g) were homogenized in 10 ml of 3% aqueous sulphosalicylic acid. 2 ml aliquot of the supernatant was mixed with an equal volume of acetic acid and acid ninhydrin and incubated for 1 h at 100°C. The reaction was terminated in ice bath and proline was extracted with 4 ml of toluene. Absorbance was determined spectrophotometrically at 520 nm (Beckman 640 D, USA) using toluene for a blank.

Protein estimation

Proteins were estimated with Bradford (1976) method. Fresh material (0.5 g) was homogenized in 1 ml phosphate buffer (pH 7.0). The crude homogenate was centrifuged at 5,000× g for 10 min. 0.5 ml of freshly prepared trichloricacetic acid (TCA) was added and centrifuged at 8,000× g for 15 min. Debris was dissolved in 1 ml of 0.1N NaOH and 5 ml Bradford reagent was added. Absorbance was recorded spectrophotometrically at 595 nm (Beckman 640 D, USA) using bovine serum albumin as a standard.

Sugar estimation

Sugar was estimated with Dey (1990) method. Leaves (0.5 g) were extracted twice with hot 90% ethanol. Ethanol extracts were then combined. The final volume of the pooled extract was made up to 25 ml with double distilled water. A suitable aliquot was taken from the extract and 1 ml 5% phenol and 5 ml of concentrated sulphuric acid were added. Final volume of this solution was made up to 10 ml by adding double distilled water. Absorbance of this solution was measured at 485 nm using a UV-Vis spectrophotometer (Beckman 640 D, USA).

Statistical analysis

Statistical analysis of the data was done following the methods of analysis of variance (ANOVA)

according to Panse and Sukhatme (1967) using Agris statistical software programme (AgRes 3.01) to confirm the validity of the data. Critical difference (CD) was calculated at 5% probability level.

RESULTS AND DISCUSSION

Survival percentage

Data referring to the effect of $NaHCO_3$ on survival are presented in Fig. 1A. A decrease of survival rate was found to be concentration-dependent. In the 1st culture the reduction of survivability was 40.9 and 44.7% in Local and Sujanpuri cultivars, respectively, at higher concentrations of T4 (59.0mM $NaHCO_3$). In the 3rd transfer the reduction of survival in cv. Local was 53%, in Sujanpuri 64.5%.

These results correspond to the findings of Vijayan et al. (2003) who showed that at 0.25% NaCl the average decline in survival of mulberry cultivars was 16.6% and at higher salinity, namely 0.75% and 1.0% NaCl, a reduction of 76.9% and 98.1%, respectively, was observed.

Shoot length (cm)

During $NaHCO_3$ stress the shoot length decreased. This decrease was higher in Sujanpuri than in Local; however, an increase in shoot length was observed in subsequent cultures but compared to the control there was a reduction with all salt concentrations (Fig. 1B). In the 4th culture with the highest salt concentration, i.e. 59mM $NaHCO_{3,,}$ the reduction of shoot length was 49.1% in Sujanpuri and 43.2% in Local.

Similarly to the present report, a negative linear relationship in shoot length was observed with an increasing concentration of salt in *Salvidora persica* reported by Ramoliya et al. (2004). Shoot length reductions of 17, 20 and 22% were recorded in *Triticum aestivum,* cv. Sarsabz at 25, 50 and 100mM of NaCl, respectively, and a decrease of 10.6, 18.5 and 21% was observed at similar levels of Na_2SO_4 compared to the control (Zaman et al. 2002). Shoot length was also significantly reduced in six olive cultivars at different concentrations of salt. Shoot length reduction ranged between 42% in Megaristiks to 78% in Mastoidis cultivars of *Olea europaea* at 200mM

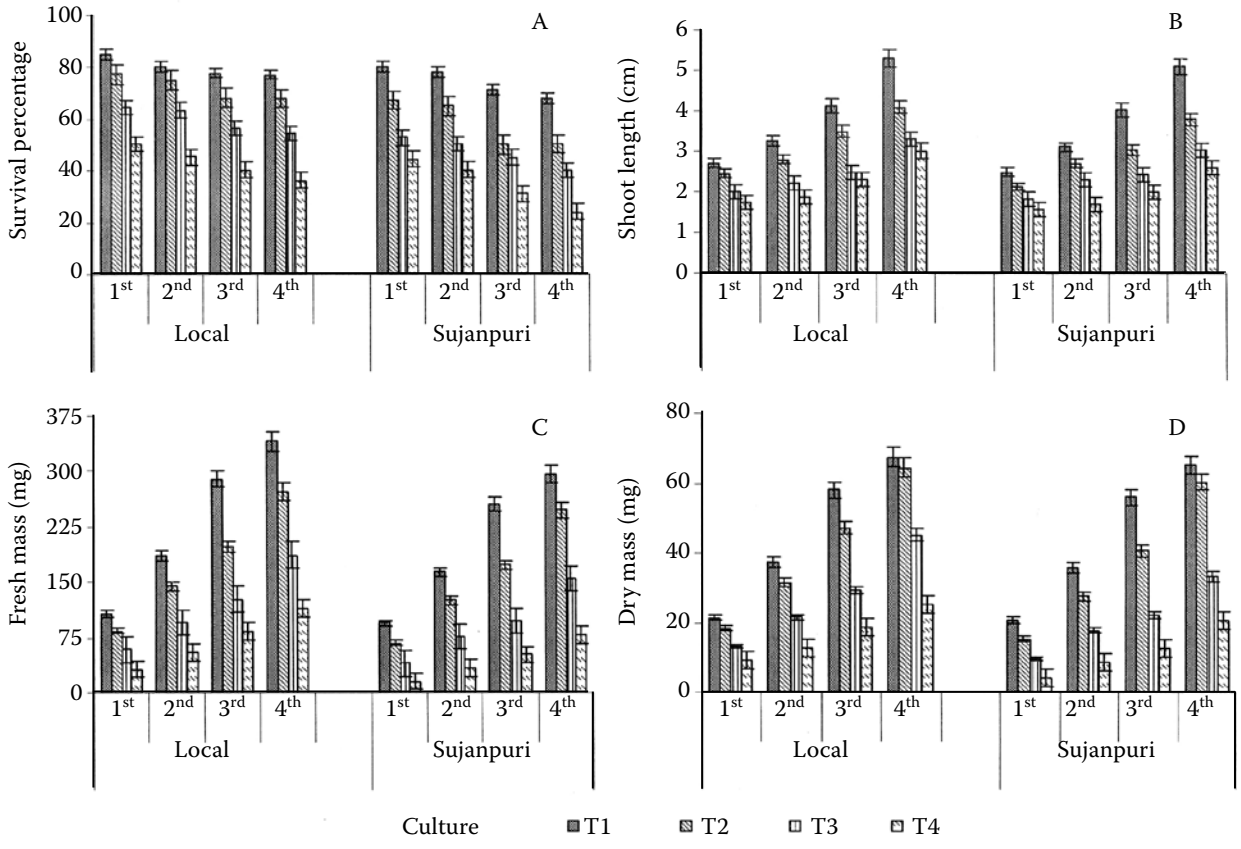

Fig. 1. Effect of $NaHCO_3$ on (A) survival, (B) shoot length (cm), (C) fresh mass (mg), and (D) dry mass (mg) in two cultivars of *Morus alba* L. Each point is the mean of three replications and the bars indicate ± SE

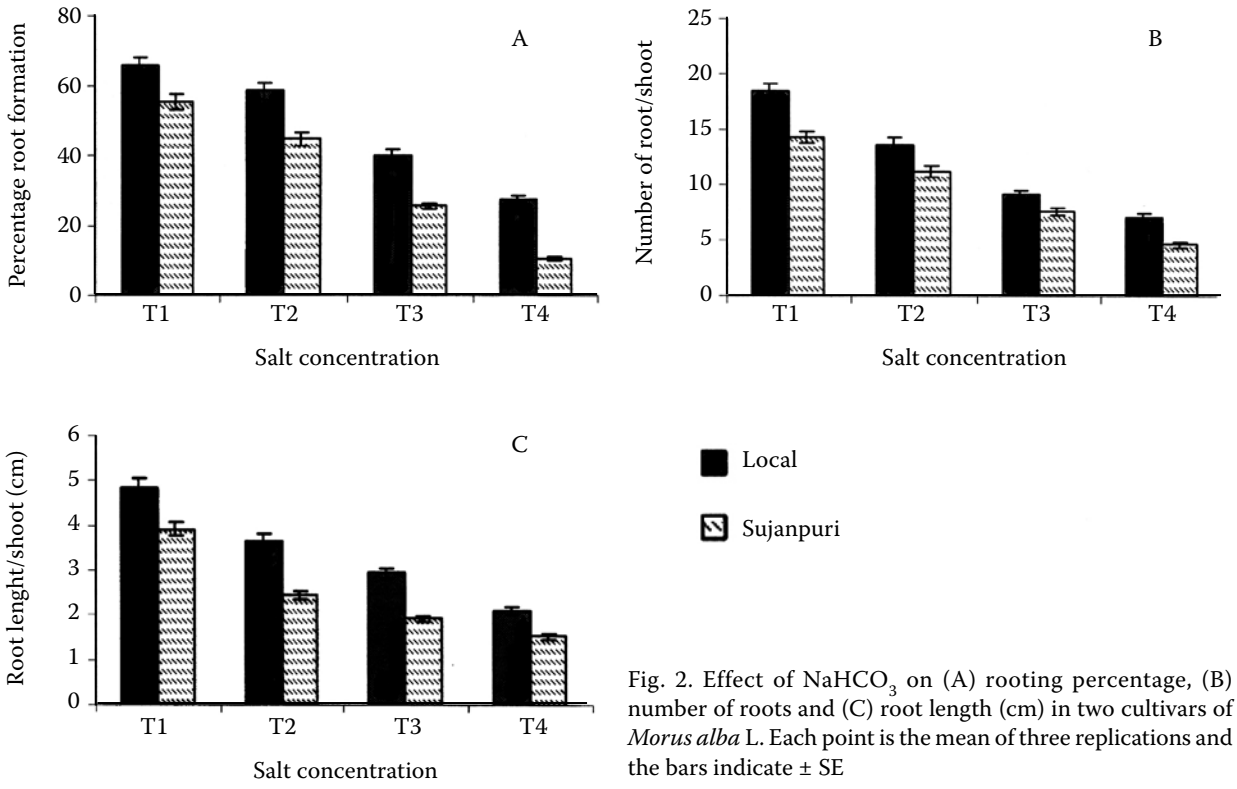

Fig. 2. Effect of $NaHCO_3$ on (A) rooting percentage, (B) number of roots and (C) root length (cm) in two cultivars of *Morus alba* L. Each point is the mean of three replications and the bars indicate ± SE

NaCl in relation to the control (Chartzoulakis et al. 2002).

Fresh and dry mass (mg)

The fresh mass decreased by 70.2% in Local and by 83.8% in Sujanpuri in the 1st culture (after 25 days) with 59.0mM $NaHCO_3$, but the subsequent cultures enhanced it (Fig. 1C).

A remarkable reduction of fresh mass of shoots developed from the axillary buds at higher salinity was reported in mulberry (Vijayan et al. 2003). However, Roussos et al. (2006) noticed that fresh mass of jojoba (*Simmondsia chinesis*) explant increased significantly over time under all stress levels, and showed a decrease only at the concentration of 169.2mM salt. Zaman et al. (2002) showed 62% and 80% reduction of shoot fresh mass at 50 and 100mM of NaCl, respectively, and 17% and 53% shoot fresh mass reduction at similar levels of Na_2SO_4 in *Triticum aestivum* cv. Sarsabz.

Fig. 1D summarizes data pertaining to the effect of $NaHCO_3$ on dry mass. The $NaHCO_3$ decline to 62.22 and 68.0% in Local and Sujanpuri, respectively.

Dry mass of *Salvadora persica* (leaf, stem and shoot) was also significantly decreased in response to increasing concentrations of salt in soil (Ramoliya et al. 2004). Biomass weight decreased with an increasing concentration of salt (40–160mM) also in *Artemisia annua* (Qureshi et al. 2005) and jojoba (*Simmondsia chinesis*) (Roussos et al. 2006). Further, Soussi et al. (1998) demonstrated that the dry mass of chick pea (*Cicer arietinum* L.) was not affected by 50 and 75mM NaCl, but at 100mM the dry matter decreased. The salinity level of 10.6/ds, under moisture and dry conditions, decreased dry matter by 34.5% and 39% in *C. rothii,* respectively (Ramoliya, Pandey 2003).

Reduced growth at higher salt levels may be associated with a marked inhibition of photosynthesis (Chartzoulakis et al. 2002). Toxic effects caused by salt accumulation might become visible at higher Na^+ and/or Cl^- concentrations and may hamper the plant growth (Chartzoulakis et al. 2002). Moreover, under salt stress conditions the K^+ concentration is severely reduced in roots as well as in leaves (Chartzoulakis et al. 2002) and this reduction in potassium ions may also be reflected in terms of reduction in different growth parameters.

Root formation

Observations of *in vitro* root formation were performed after the 4th culture (100 days) and the results are presented in Fig. 2A. Percentage of root formation declined in both cultivars due to salt stress, but this effect was more prominent in cv. Sujanpuri than in Local. A significant decrease, namely 58% and 81.4%, was observed in Local and Sujanpuri, respectively, at the stress level of 59mM $NaHCO_3$.

Number of roots per plantlet

Fig. 2B represents results of the effect of $NaHCO_3$ on number of roots per plantlet. At the concentration of 59mM $NaHCO_3$, the number of roots decreased to 61.3 and 68.0% in Local and Sujanpuri, respectively. A detrimental effect of different concentration levels of $NaHCO_3$ on the number of roots per plantlet was reported to be directly related to salt concentration and exposure time.

Root length (cm)

The effect of different concentrations of $NaHCO_3$ on root length was studied after the 4th culture. A decrease of 55.2% in root length of Local and 60.8% in Sujanpuri was observed with 59mM $NaHCO_3$ (Fig. 2C).

A linear decrease of root length was observed in increasing concentrations of salt in *Salvadora persica* (Ramoliya et al. 2004). The root length decreased by 19.7% in 10.6 ds under moisture conditions, and by 27.4% in 10.6/ds under dry conditions in *Cordia rothii* (Ramoliya, Pandey 2003). Zaman et al. (2002) showed a root length reduction of 15, 35 and 47% at 25, 50 and 100mM NaCl, respectively, and 8, 12 and 30%, at similar levels of Na_2SO_4, in *Triticum aestivum* cv. Sarsabz. For root formation, nutrient salts contained in the medium in a balanced and optimum amount may have a dramatic effect both on rooting percentage and root number per plantlet (Bhojwani, Razdan 1996). Roots are reported to be among the first organs that show more sensitivity under salt stress (Muscolo et al. 2003). The presence of sodium in $NaHCO_3$ might have hindered the uptake of other essential nutrients and affected root parameters.

Pigments

Chlorophyll a, b and total

In the 1st culture at T4 concentration of $NaHCO_3$ (pH 8.2) chlorophyll *a* declined to 48.2%, chlorophyll *b* to 57.1%, and total chlorophyll to 51.6% in Sujanpuri; Local showed a decrease of 40% in chlorophyll *a*, 50% in chlorophyll *b* and 43% in total chlorophyll (Table 1). Cultivar Local in the 4th culture did not show any significant effect of $NaHCO_3$ stress, whereas a little decrease was observed in Sujanpuri.

Our results are in accordance with several reports on decreased contents of chlorophyll by salt stress as reported in number of glycophytes (Agastian et al. 2000). Harinasut et al. (2000) reported a decrease in chlorophyll *a*, *b* and total in mulberry with

Table 1. *In vitro* effect of different concentrations (T1, T2, T3, T4) of $NaHCO_3$ on chlorophyll *a*, *b* and total content (mg/g fw) in two cultivars of *Morus alba* L. Data are mean values of three independent replicates ± S.D.

Culturing	Parameters	Local cultivar				Sujanpuri cultivar				CD at 5%		
		T1	T2	T3	T4	T1	T2	T3	T4	V	T	VT
1st culture	chl. *a*	0.65 ± 0.03	0.54 ± 0.02	0.41 ± 0.05	0.39 ± 0.001	0.58 ± 0.04	0.44 ± 0.02	0.39 ± 0.001	0.30 ± 0.02	0.0071	0.0101	0.021
	chl. *b*	0.28 ± 0.001	0.19 ± 0.02	0.17 ± 0.01	0.14 ± 0.005	0.35 ± 0.01	0.30 ± 0.01	0.21 ± 0.001	0.15 ± 0.001	0.041	0.047	0.060
	total chl.	0.93 ± 0.04	0.78 ± 0.03	0.61 ± 0.04	0.53 ± 0.04	0.93 ± 0.04	0.74 ± 0.07	0.6 ± 0.03	0.45 ± 0.05	0.029	0.037	0.051
4th culture	chl. *a*	0.67 ± 0.03	0.58 ± 0.05	0.43 ± 0.04	0.40 ± 0.07	0.59 ± 0.04	0.47 ± 0.04	0.43 ± 0.06	0.30 ± 0.04	0.009	0.022	0.025
	chl. *b*	0.34 ± 0.07	0.24 ± 0.001	0.21 ± 0.01	0.16 ± 0.002	0.40 ± 0.01	0.34 ± 0.001	0.24 ± 0.002	0.16 ± 0.03	0.029	0.040	0.053
	total chl.	1.0 ± 0.05	0.82 ± 0.03	0.64 ± 0.03	0.56 ± 0.009	0.99 ± 0.04	0.81 ± 0.06	0.67 ± 0.04	0.46 ± 0.07	0.029	0.042	0.058

For Table 1 and 2: V – cultivars, T – concentrations, V × T – cultivar × concentrations
T1 – control (pH 5.8), T2 = 3.57mM (pH 6.2), T3 = 20mM (pH 7.2), T4 = 59mM (pH 8.2)

increasing concentrations of salt in the media. Total chlorophyll content decreased significantly with the NaCl treatment in *Aegiceras corniculatum* (Parida et al. 2004). However Wang and Nil (2000) found out that chlorophyll content increased under conditions of salt stress in *Amaranthus tricolor*. The inhibitory effects of salt on chlorophylls could be caused by suppression of specific enzymes responsible for the synthesis of green pigments (Strogonove et al. 1970). A decrease in the chlorophyll content may be attributed to the chlorophyllase activity (Sudhakar 1997). A lower reduction of chlorophyll pigments in the tolerant genotypes may be responsible for a higher dry matter accumulation (Garg, Singla 2004).

Proline content

Results related to the effect of $NaHCO_3$ on proline content are summarized in Table 2. Proline content increased to 89.2% and 85.5% in Local and Sujanpuri cultivars, respectively, at concentration T4 in the 1st culture and the subsequent culturing did not show any significant increase.

Our results of increased proline with $NaHCO_3$ treatments correspond to the findings of Agastian et al. (2000) who reported an increase in proline content with increasing concentration of salt in mulberry genotypes. Harinasut et al. (2000) showed that proline content in leaves of mulberry increases 11 times over control with 150mM NaCl conditions. However, Parida et al. (2004) reported decreasing proline content in leaves of *Aegiceras corniculatum* during NaCl exposure. A positive correlation between magnitude of free proline accumulation and salt tolerance was suggested as an index for determining salt tolerance potentials between cultivars (Ramanjula, Sudhakar 2001). Proline improves the salt tolerance by protecting protein turnover mechanism and by upregulating stress protective proteins (Thakur, Sharma 2005).

Proline interacts with enzymes to preserve protein structure and activities, reduces enzyme denaturation caused due to heat, salt stress etc., and acts as a reserve source of carbon, nitrogen and energy during recovery from stress (Sairam, Tyagi 2004).

Protein content

Increase of protein accumulation was 53.9% in cultivar Local and 50.4% in Sujanpuri with the treatment T3 (20mM $NaHCO_3$) in the 1st culture, while a slight increase of 54.5% and 53% was observed in Local and Sujanpuri, respectively, in the 4th culture

Table 2. *In vitro* effect of different concentrations (T1, T2, T3, T4) of $NaHCO_3$ on proline (µg/g fw), protein (mg/g fw) and soluble sugar (mg/g fw) content in two cultivars of *Morus alba* L. Data are mean values of three independent replicates ± S.D.

Culturing	Parameters	Local cultivar				Sujanpuri cultivar				CD at 5%		
		T1	T2	T3	T4	T1	T2	T3	T4	V	T	VT
1st culture	proline	8.97 ± 0.2	27.91 ± 0.9	28.34 ± 0.8	83.51 ± 1.8	8.70 ± 0.2	25.02 ± 0.8	40.34 ± 1.3	60.2 ± 1.5	1.31	1.95	2.77
	protein	5.5 ± 0.1	9.07 ± 0.2	11.95 ± 0.3	11.86 ± 0.2	4.98 ± 0.1	6.77 ± 0.1	10.05 ± 0.6	9.99 ± 0.2	0.61	0.77	1.04
	sugar	3.21 ± 0.09	5.89 ± 0.1	8.75 ± 0.2	8.69 ± 0.2	2.85 ± 0.0	4.30 ± 0.1	7.15 ± 0.1	7.02 ± 0.1	0.44	0.65	0.81
4th culture	proline	9.05 ± 0.2	29.51 ± 0.9	51.71 ± 1.5	85.02 ± 1.9	8.80 ± 0.2	27.32 ± 0.8	43.04 ± 1.4	67.11 ± 0.1	2.20	3.10	4.30
	protein	5.71 ± 0.2	9.2 ± 0.2	12.57 ± 0.3	12.51 ± 0.4	5.27 ± 0.1	6.9 ± 0.1	11.23 ± 0.3	11.09 ± 0.2	0.83	1.2	1.54
	sugar	3.41 ± 0.1	6.01 ± 0.1	9.7 ± 0.2	9.63 ± 0.3	2.97 ± 0.06	5.61 ± 0.1	7.9 ± 0.2	7.81 ± 0.2	0.65	0.91	1.35

(3rd transfer) at the same concentration. At higher concentrations of T4 (59mM $NaHCO_3$) protein accumulation declined in both cultivars (Table 2).

Our results of increasing protein in low concentration of salt and decreasing at high concentration of salt correspond with the findings of Agastian et al. (2000) who showed that the content of soluble protein increased at low salinity and decreased at higher salinity (8 and 12 ms/cm), irrespective of mulberry genotypes.

At lower levels of NaCl (75mM NaCl) there was an increase in protein content but higher concentrations (150–300mM NaCl) caused it to decline in shoots and roots of *Pancratium maritimum* (Khedr et al. 2003). This suggests that the initial response to salt-developed water stress involves an increased protein synthesis that is prevented when the stress becomes too severe (Khedr et al. 2003). Highly decreased protein contents were reported in mangrove (*Bruguiere parviflora*) by Parida et al. (2002) in response to salt stress.

Protein degradation might be the result of an increased activity of the protease or other catabolic enzymes that are activated under salt stress (Ramanjula et al. 1994).

Soluble sugar content

Table 2 deals with the results related to the effect of $NaHCO_3$ on soluble sugar content. An increase of 63.3% and 60.1% was observed in Local and Sujanpuri cultivars, respectively, in the 1st culture at the concentration of T3 (20mM $NaHCO_3$), while in the 4th culture (3rd subculture) an increase of 64.8% in cultivar Local and 62.4% in cultivar Sujanpuri was noticed at the same concentration. A decrease in sugar content was observed at the higher concentration T4 (59mM $NaHCO_3$) in both cultivars of *M. alba*.

Agastian et al. (2000) reported that low salt concentrations induce soluble sugars, and at higher concentrations a decrease was observed in mulberry genotypes. Ashraf and Tufail (1995) determined that total soluble sugar content increases with the increase in salt concentration in sunflower. Carbohydrates such as sugars (glucose, fructose, sucrose, fructans) and starch accumulate under salt stress in true mangrove, *Bruguiera parviflora* (Parida et al. 2002). Their major functions are osmoprotection, osmotic adjustment, carbon storage and radical scavenging (Parida, Das 2005).

CONCLUSIONS

A lot of work has been done on salinity stress on plants; yet, studies on alkalinity stress are very scarce. The results obtained in the present study revealed that screening of salt tolerant genotypes of mulberry by tissue culture can be employed for the reclamation of alkalinity-affected land. Tissue culture is the only technique to bring such variability in mulberry genotypes. An increase in osmotic solutes helps the mulberry plant to withstand the environmental challenges.

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Selekce kultivarů *Morus alba* (Local a Sujanpuri) tolerantních vůči $NaHCO_3$ v podmínkách *in vitro* v reakci na morfologické a biochemické parametry

ABSTRAKT: V *in vitro* experimentech byl zkoumán dopad stresu způsobeného $NaHCO_3$ (alkaličnost) na mladé sazeničky dvou kultivarů *Morus alba* (Local a Sujanpuri), vypěstované z nodálních explantátů. Pro rozmnožování výhonků bylo použito 2,5 mg/l 6-benzylaminopurinu (BAP) s 0,3 mg/l kyseliny giberelové (GA_3), tvorba kořenů byla iniciována pomocí 1,0 mg/l kyseliny indolbutyrické (IBA). $NaHCO_3$ byla přidána do kultivačního média ve třech koncentracích: 3,57, 20 a 59mM, jež odpovídaly zvýšení pH na 6,2, 7,2 a 8,2. Zvýšená koncentrace soli ovlivnila růstové parametry, následná péče tyto vlivy podpořila. Byla provedena analýza obsahu některých látek (prolin, proteiny, cukry, chlorofyl) v pěstované biomase; její výsledky indikují zvýšení obsahu prolinu, proteinů a cukrů; nicméně vyšší koncentrace $NaHCO_3$ způsobují jejich pokles. Snížení chlorofylu bylo pozorováno ve všech stresových režimech.

Klíčová slova: $NaHCO_3$; biochemické parametry; *in vitro* selekce; *Morus alba*

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